Example 2 - Physical Characterization of Unmodified and Surface-Modified GOAM

The determination of the size distribution, concentration, and size fractionation of the synthesized GOAM is accomplished via Coulter counter analysis. In addition, optical microscopic images (Bausch & Lomb) are used to verify the size and conformation of GOAM.

Example 3 - Acoustic Simulations

Two different simulation approaches are required to describe the characteristics of the acoustic driving forces on the developed microsphere as well as the acoustic propagation of the scattered ultrasonic energy. One approach uses boundary element method (BEM) modeling to describe the acoustic behavior of the microsphere. Finite difference time domain modeling (FDTD) is used to examine the backscattering properties of the reflected acoustic pressure waves.

Example 4 - Ultrasound Characterization of Unmodified and Surface-Modified GOAM

General Procedures

The *in vitro* ultrasonic characteristics of synthesized unmodified or surface-modified GOAM are determined at different concentrations of Gd_2O_3 at constant temperatures. The following characteristics are determined: bubble size-distribution, life time, effect of ultrasound machine power, effect of suspension condition - dilution and carrier medium, attenuation (as a function of frequency), sound velocity, normalized backscatter coefficient, and scattering.

The acquisition of all ultrasound signals is accomplished using an Aloka 5500 PHD RF machine using two different ultrasound transducers (Aloka UST 5539 10 MHz linear

Banner & Witcoff, Ltd.
Ten South Wacker Drive
Chicago, IL 60606-7407

small parts transducer and Aloka UST 9119 2-5 MHz curvilinear abdominal transducer). The Aloka 5500 PHD RF stores simultaneously, in real time, multiple frames of RF data from all the transducer elements as well as the corresponding B-mode images. These data are then ported to a PC (Intel 800 MHz PIII) for off-line analyses of the independent RF element data as well as the associated B-mode images.

Forward scattering data acquisition is accomplished using a high performance needle hydrophone data acquisition system (Precision Acoustics, Ltd. Digital Acquisition System) with a 0.04 mm 9 micron PVDF probe). These data are stored on a personal computer.

A Bausch and Lomb optical microscope is used to acquire digitized optical images of the microbubbles. Digital image acquisition is accomplished using a Sony CCD camera (Model 1250) linked to a Pinnacle Systems video frame grabber board (Miro DC30 plus) and a PC (Intel 800 MHz PIII) and stored on the hard drive. All images are acquired in RGB mode with an image size of 608 x 456 pixels. Specially developed image capture software (Capture ©,Watkin, 1998-2001) permits real time image capture at 30 fps.

Both *in vitro* and *in vivo* B-mode imaging data are simultaneously recorded using a separate computer acquisition system. Digital image acquisition is accomplished using a Pinnacle Systems video frame grabber board (Miro DC30 plus) and a PC (Intel 800 MHz PIII) and stored on the hard drive. All images are acquired in RGB mode with an image size of 608 x 456 pixels. Specially developed image capture software (Capture ©,Watkin, 1998-2001) permits real time image capture at 30 fps. This acquisition system is connected directly to the color video output of the Aloka 5500 PHD RF machine.

Banner & Witcoff, Ltd.
Ten South Wacker Drive
Chicago, IL 60606-7407

An acrylic imaging tank, (25 cm x 15 cm x 15 cm) with a 10 cm x 10 cm thin membrane window at one end for acoustic monitoring is filled with freshly degassed, de-ionized water at constant temperature (22°C). Ready-to-use cellulose dialysis tubes (240 μm) are fixed across the width of the imaging tank at 1 cm, 2 cm and 3 cm depths from the imaging window. These tubes are filled with the contrast media selected for each experiment. Rinsing protocols are used following the injection of each contrast media.

Bubble size-distribution

Two different methods are used to determine the size and distribution of the unmodified and surface-modified GOAM. An optical microscope is used for optical verification of the sizes and distributions of the microbubbles at 10x and 40x power. Calibration is provided by precision graticule slides.

More precise bubble sizing and distribution data is determined using a Beckman-Coulter Multisizer Z2.

GOAM Life Time

The echogenicity of unmodified and surface-modified GOAM is tested over an extended period of time to assess the time period during which GOAM remain stable within a specially constructed imaging vial. A small imaging vial with a thin acoustic membrane is used for this purpose. Ultrasound imaging acoustic power is fixed at a mechanical index of 0.7. A fixed concentration of microbubbles is used. The concentration is in the linear range of the backscatter/concentration plot.

Banner & Witcoff, Ltd.
Ten South Wacker Drive
Chicago, IL 60606-7407